

Slow Components of Potassium Tail Currents in Rat Skeletal Muscle

KURT G. BEAM and P. LYNN DONALDSON

From the Department of Physiology and Biophysics, University of Iowa School of Medicine, Iowa City, Iowa 52242

ABSTRACT The kinetics of potassium tail currents have been studied in the omohyoid muscle of the rat using the three-microelectrode voltage-clamp technique. The currents were elicited by a two-pulse protocol in which a conditioning pulse to open channels was followed by a test step to varying levels. The tail currents reversed at a single well-defined potential (V_K). At hyperpolarized test potentials (-100 mV and below), tail currents were inward and exhibited two clearly distinguishable phases of decay, a fast tail with a time constant of 2–3 ms and a slow tail with a time constant of ~ 150 ms. At depolarized potentials (-60 mV and above), tail currents were outward and did not show two such easily separable phases of decay, although a slow kinetic component was present. The slow kinetic phase of outward tail currents appeared to be functionally distinct from the slow inward tail since the channels responsible for the latter did not allow significant outward current. Substitution of Rb for extracellular K abolished current through the anomalous (inward-going) rectifier and at the same time eliminated the slow inward tail, which suggests that the slow inward tail current flows through anomalous rectifier channels. The amplitude of the slow inward tail was increased and V_K was shifted in the depolarizing direction by longer conditioning pulses. The shift in V_K implies that during outward currents potassium accumulates in a restricted extracellular space, and it is suggested that this excess K causes the slow inward tail by increasing the inward current through the anomalous rectifier. By this hypothesis, the tail current slowly decays as K diffuses from the restricted space. Consistent with such a hypothesis, the decay of the slow inward tail was not strongly affected by changing temperature. It is concluded that a single delayed K channel is present in the omohyoid. Substitution of Rb for K has little effect on the magnitude or time course of outward current tails, but reduces the magnitude and slows the decay of the fast component of inward tails. Both effects are consistent with a mechanism proposed for squid giant axon (Swenson and Armstrong, 1981): that (a) the delayed potassium channel cannot close while Rb is inside it, and (b) that Rb remains in the channel longer than K.

Address reprint requests to Dr. Kurt G. Beam, Dept. of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/83/04/0513/18 \$1.00 513
Volume 81 April 1983 513–530

INTRODUCTION

Potassium tail current kinetics in vertebrate skeletal muscle are complex, especially at hyperpolarized potentials where the tail currents are inward. Thus, when a depolarizing pulse to activate K channels is followed by a hyperpolarizing pulse, the tail current decays with a fast and slow phase in frog sartorius (Adrian et al., 1970*b*), rat iliacus, and rat soleus (Duval and Léoty, 1978, 1980*a, b*) muscles. The generally accepted explanation for the fast phase is that it represents the closing of ordinary, voltage-activated, delayed-rectifier channels opened by the conditioning pulse. Several alternative explanations have been proposed for the slow component of the inward tail, including (*a*) voltage activation of slow K channels (Adrian et al., 1970*b*); (*b*) calcium activation of K channels (cf. Barrett et al., 1981); and (*c*) potassium accumulation (Duval and Léoty, 1980*b*). Knowing whether one or more of these processes occurs is essential for a mechanistic understanding of outward currents in any particular muscle preparation. For example, in the preceding paper (Beam and Donaldson, 1983) we described the quantitative and qualitative effects of temperature on the kinetics of outward current in rat skeletal muscle. One interpretation of these effects is that temperature is affecting the gating mechanism of a single species of K channel. Alternatively, temperature may have a differential effect on two or more species of channel.

Duval and Léoty (1980*b*) have reported a differential effect of 4-aminopyridine (4-AP) on outward currents in rat soleus and iliacus muscle. They found that after a depolarizing step 4-AP causes a time-independent reduction of outward current in the iliacus, but causes a reduction of outward current in soleus which is greater at early times than late. This result is suggestive of pharmacologically distinct K channels in the two muscle types and possibly two kinds of channels in soleus. An additional finding was that glycerol shock eliminated the slow inward tail in iliacus but not in soleus. Glycerol shock also reduced K accumulation (as judged by the time-dependent shift in the potassium equilibrium potential). Based on these observations, Duval and Léoty (1980*b*) suggested that the soleus possesses two distinct, voltage-activated, delayed K channels, that the iliacus possesses only one delayed K channel, and that K accumulation is responsible for the slow inward tail in iliacus. No explanation was offered, however, for precisely how accumulation would cause the slow tail. Moreover, the glycerol shock experiment does not allow a clear distinction between the possibilities that the slow tail in iliacus arises from accumulation or from the presence of a slow K channel predominantly located in the transverse tubules. Finally, it is very difficult to compare currents in a single fiber before and after glycerol shock. Such a comparison is important given the possibility that in some preparations accumulation and slow K channels may both contribute to the slow tail. For these reasons we have re-examined slow tail currents in the omohyoid, a fast-twitch muscle (Müntener et al., 1980). In particular, we used pulse protocols and ion substitutions designed to pinpoint the source of various components of tail current in single fibers. A preliminary account of some of our findings has appeared in abstract form (Donaldson and Beam, 1981).

METHODS

The omohyoid muscle of the rat was investigated using the three-microelectrode voltage-clamp technique (Adrian et al., 1970a). Details of experimental methods are given in the preceding paper (Beam and Donaldson, 1983). In some experiments, potassium, which was normally present at 5 mM in the experimental bathing medium (solution B, Table I, Beam and Donaldson, 1983), was replaced with 5 mM rubidium. Where necessary, a fast sample rate was used during the early portion of a record and a slow rate was used for the later portion in order to conserve computer memory and still obtain good temporal resolution of both rapid and slow phases of the current record.

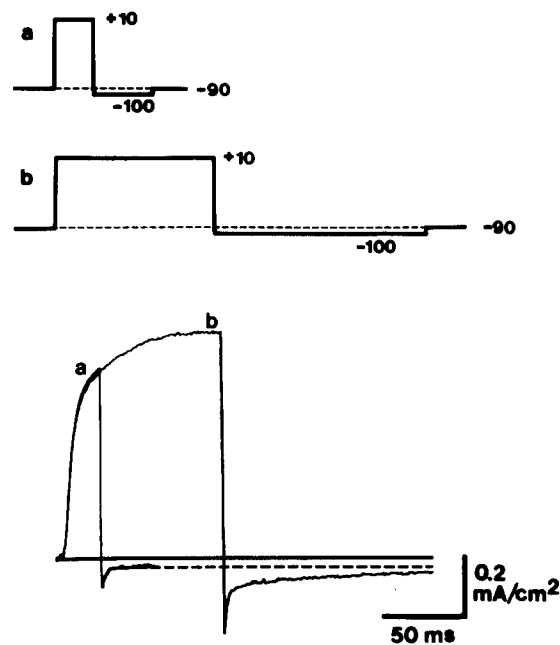


FIGURE 1. Effect of conditioning pulse duration on tail current kinetics. The upper panel of the figure shows the pulse protocols and the lower panel shows the resulting currents. After a 25-ms conditioning pulse to +10 mV (*a*), the inward tail current at -100 mV decays as a single exponential. After a 100-ms conditioning pulse (*b*), the inward tail current at -100 mV shows a rapid decay as in *a*, followed by a much more slowly decaying tail current. (The horizontal dashed line indicates the current that flowed at -100 mV in the absence of a prior conditioning pulse.) $T = 14^{\circ}\text{C}$. Muscle 43-2: $l, l' = 168, 70 \mu\text{m}$; $r_i = 11.1 \text{ M}\Omega/\text{cm}$; $\lambda = 601 \mu\text{m}$; $C = 7.6 \mu\text{F}/\text{cm}^2$.

RESULTS

Tail Current Kinetics and Conditioning Pulse Duration

Fig. 1 illustrates the currents observed when a fiber was repolarized to -100 mV after a 25- (*a*) or 100-ms (*b*) conditioning pulse to +10 mV. After the shorter conditioning pulse, the current decays as a single exponential ($\tau = 2.4$

ms) which represents the closing of K channels opened during the conditioning pulse. The current does not decay to zero but instead reaches a steady-state level representing inward current through the anomalous rectifier. In contrast, a 100-ms prepulse produced a tail current (*b*) that decays as the sum of two exponentials, the fast exponential with a time constant near that of the tail current in *a*, and an additional slow exponential ($\tau = 134$ ms). The dependence of the amplitude of this slow inward tail on the duration of the conditioning pulse was somewhat variable from fiber to fiber. For example, after a 25-ms conditioning pulse a slow tail was not detectable at -100 mV in the fiber illustrated in Fig. 1, but was detectable in the fiber illustrated in Fig. 2. In addition to displaying fiber-to-fiber variability in the time course of development, the slow tail also showed considerable fiber-to-fiber variability in its rate of decay. In eight fibers the time constant of the slow tail at -120 mV ranged from 48 to 205 ms with a mean (\pm SD) of 130 ± 54 ms ($T \cong 15^\circ\text{C}$, 25-ms conditioning pulse to $+10$ mV).

Lengthening the conditioning pulse not only increased the amplitude of the slow tail but also slowed its decay. For example, in one fiber, changing the length of the conditioning pulse ($+10$ mV) from 25 to 100 ms increased 1.5-fold the amplitude of the slow tail at -120 mV and slowed its time constant from 114 to 138 ms.

The time constant of the slow inward tail in the omohyoid is in the range reported by Adrian et al. (1970*b*) for a slow component of current decay in frog muscle under similar conditions. Adrian et al. hypothesized that the slow decay represents the closing of a second type of K channel opened by longer conditioning pulses but not by shorter ones. Seeming to support such a hypothesis in the omohyoid is the observation that between 25 and 100 ms the outward current is slowly increasing (Fig. 1). This phase of slowly increasing current cannot be fit by the same n^4 kinetics that account for the initial rising phase of current (Beam and Donaldson, 1983) and may therefore represent the activation of the same slowly gated potassium channels whose closing is manifested in the slow tail. A hypothesis similar to that of Adrian et al. is to suppose that the slow tail represents the closing of calcium-activated K channels. Such channels have been shown to be present in cultured embryonic rat muscle (Barrett et al., 1981; Pallotta et al., 1981).

An Accumulation Hypothesis for the Slow Inward Tail

As an alternative to the notion that slow voltage- or calcium-activated K channels cause the slow tail, the following hypothesis is suggested. During outward current flow, potassium ions accumulate within a restricted extracellular space of unknown anatomic identity (see below). The accumulated potassium increases inward current through the anomalous rectifier at any given voltage. This extra current, seen as the slow inward tail, decays in time as the excess potassium is removed from the restricted space. An attractive aspect of such an accumulation hypothesis is that it accords more easily with the observed fiber-to-fiber variability of the properties of the slow tail than does a separate-channel hypothesis. In particular, one might reasonably expect

far greater variability in the buildup and removal of extracellular K than in the opening and closing kinetics of a distinct species of delayed K channel. An accumulation hypothesis also fits nicely with the weak temperature dependence of the time constant of the slow inward tail. This time constant decreased from 153 ± 54 ms (mean \pm SD, $N = 8$) at 15°C to 94 ± 30 ms ($N = 5$) at 21°C . This change in time constant corresponds to a Q_{10} of 1.7, a value not much greater than that expected for diffusion.

The experiments described below were designed to address three questions bearing on the accumulation hypothesis: (a) Do potassium tail currents have a single well-defined reversal potential? (b) Is the slow inward tail current carried by the anomalous rectifier? (c) Is the same mechanism responsible for the slow kinetic component of outward tail currents that is responsible for the slow inward tail?

Does Tail Current Reverse at a Single Potential?

As already described, the inward tail current at hyperpolarized potentials shows two distinct phases of decay (Fig. 1). The outward tail current at more depolarized potentials does not show two such clearly distinguishable phases, although a kinetic component too slow to be fit by simple exponential kinetics is present (cf. Fig. 6, Beam and Donaldson, 1983). The behavior of fast and slow phases of tail current above and below the reversal potential are illustrated in Fig. 2. This fiber had a reversal potential very near -80 mV. At

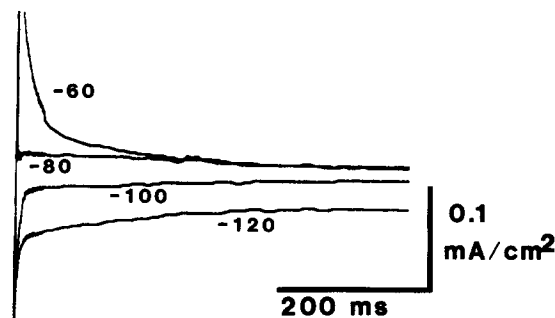


FIGURE 2. Tail current reverses at a single potential. After a 25-ms step to $+10$ mV (current not shown), voltage was stepped to the indicated test potentials. For this fiber, the test pulse to -80 mV happened to lie very near the reversal potential for potassium current. The current at -80 mV remains uniformly near zero at all times, which argues against the presence of two or more separate currents with distinct reversal potentials. $T = 14.3^\circ\text{C}$. Muscle 52-2: $l, l' = 140, 50$ μm ; $r_i = 13.5$ $\text{M}\Omega/\text{cm}$; $\lambda = 606$ μm ; $C = 7.5$ $\mu\text{F}/\text{cm}^2$.

-100 and -120 mV the tail currents are inward and display both the fast and slow phase; at -60 mV the tail current is outward; and at -80 mV the current remains uniformly near zero. If it were to be supposed that the tail current, $I(t)$, is the sum of currents through two distinct kinds of channels, then $I(t)$ at any particular voltage (V) is given by the expression

$$I(t) = g_{\text{fast}} (V - V_{\text{fast}}) + g_{\text{slow}} (V - V_{\text{slow}}), \quad (1)$$

where g_{fast} and g_{slow} are the conductances associated with the fast and slow components of the tail and V_{fast} and V_{slow} are the respective reversal potentials. With this hypothesis, if V_{slow} were different from V_{fast} , then a single potential at which the tail current remained uniformly close to zero should not exist. However, since a single reversal potential does exist, V_{fast} and V_{slow} must be very similar.

The fast phase of the tail current decays more slowly at depolarized potentials than at hyperpolarized ones. For example, the fast phase of the tail current in Fig. 2 had a time constant of 2.4 ms at -120 mV and a time constant of 18.5 ms at -60 mV. By contrast, the slow kinetic phase of outward tail currents at depolarized potentials decayed more rapidly than the slow inward tail at hyperpolarized ones (in Fig. 2 the slow inward tail at -120 mV had a time constant of 170 ms compared with 100 ms for the slow kinetic phase at -60 mV). If the slow inward tail and the slow kinetic phase of outward tails represented the closing rate of a distinct delayed K channel, one would have to conclude that the voltage dependence of this closing rate is atypical of voltage-activated K channels. On this basis, then, it appears that the slow kinetic phase of outward tail currents may represent a different process than the slow inward tail. This issue is discussed at greater length later.

The reversal potential (V_K) of potassium tail currents in the omohyoid shifts in the positive direction for longer conditioning pulses. Similar shifts have been described in frog muscle (Adrian et al., 1970a) and in rat iliacus and soleus muscles (Duval and Léoty, 1980b). Table I summarizes data on the shift of V_K in eight omohyoid muscles. The shift in V_K suggests that during outward currents, K accumulates in a restricted extracellular space. An estimate of the effective volume of this space is obtained by dividing the change in K concentration, calculated from the shift in V_K , by the total amount of K that leaves the fiber, as calculated from the integral of outward current. This calculated volume averaged $12 \pm 6\%$ of the total fiber volume, which is somewhat smaller than the value reported for frog muscle (Adrian et al., 1970a). As in the frog, the calculated volume of the restricted space is much too large to be that of the transverse tubular system alone, since its volume is $\sim 0.3\%$ that of the total fiber (Luff and Atwood, 1971; Eisenberg and Kuda, 1975).

Rectification of Slow Inward Tail Current

The shift in V_K supports one part of the accumulation hypothesis, the presence of a K accumulation space in the omohyoid. The experiment shown in Fig. 3 tests the other part of the hypothesis, the involvement of the anomalous rectifier. Trace *a* of Fig. 3 illustrates a tail current at -50 mV after a 25-ms conditioning pulse to $+10$ mV; this tail current is outward because V_K for this fiber was -85 mV. Trace *c* is the tail current at -120 mV, which shows the rapid and slow phases of decay already described. In pulse protocol *b*, the step to -120 mV lasted for 20 ms, a time sufficiently long that the decay of the

fast tail is complete, but sufficiently short that very little decay of the slow tail has occurred. After this 20-ms interval, the potential was stepped up to -50 mV. No outward current is present after the step to -50 mV, as would have been expected if the channels responsible for the slow tail allowed both inward

TABLE I
CALCULATION OF K^+ ACCUMULATION SPACE

	ΔV_K	$[Q_K]$	Restricted space (fraction of fiber volume)
	<i>mV</i>	<i>mM</i>	
	5	0.20	0.19
	10	0.11	0.05
	14	0.38	0.11
	15	0.39	0.10
	15	0.90	0.23
	17	0.24	0.05
	19	0.65	0.12
	20	0.44	0.08
$\bar{X} \pm SD$	14.4 \pm 4.9	0.41 \pm 0.26	0.12 \pm 0.06

Potassium accumulation in eight different fibers at $\sim 15^\circ\text{C}$. ΔV_K is the depolarizing shift in V_K , the I_K reversal potential, which was observed upon increasing the duration of the ($+10$ mV) conditioning pulse from 25 to 100 ms. The quantity $[\Delta K]_o$, the increase in apparent extracellular K^+ concentration necessary to cause the observed change in V_K , was calculated according to:

$$[\Delta K]_o = K_o(e^{\Delta V_K(F/RT)} - 1),$$

where K_o is the bulk extracellular K^+ concentration and F/RT has the usual meaning. (For simplicity, this calculation assumes that no K^+ accumulates during the first 25 ms of current flow and that the potassium channel is purely K^+ selective.) $[Q_K]$, the increased concentration of K^+ which would have resulted if all the outward current had accumulated in a space having a volume equal to that of the fiber, was calculated according to:

$$[Q_K] = (4/Fd) \int_{25 \text{ ms}}^{100 \text{ ms}} I_K(t),$$

where $I_K(t)$ is the potassium current density ($\mu\text{A}/\text{cm}^2$), F is Faraday's number, and d is the fiber diameter. The restricted space is the ratio $[Q_K]/[\Delta K]_o$.

and outward current. Specifically, if the channels responsible for the slow inward tail exhibited a linear current-voltage relationship, the magnitude of the outward current should have been about the same as that of the slow tail at -120 mV just before the step to -50 mV, since the magnitude of the driving force is nearly identical at -120 and -50 . The failure of such an outward current to be observed is consistent with the hypothesis that the slow tail at -120 mV represents current flowing through a channel that only allows inward current. An obvious candidate for such an inwardly rectifying channel is the anomalous rectifier.

As already mentioned, outward tail currents, like the one illustrated in Fig.

3 at -50 mV, do not decay with a simple time course, but instead decay with a rapid phase followed by a slower one. The experiment of Fig. 3 argues that the slow kinetic phase of outward tail current is unrelated to the slow inward tail current. In particular, the current in trace *b* of Fig. 3 demonstrates that 20 ms at -120 mV resets to zero the process responsible for the slow component

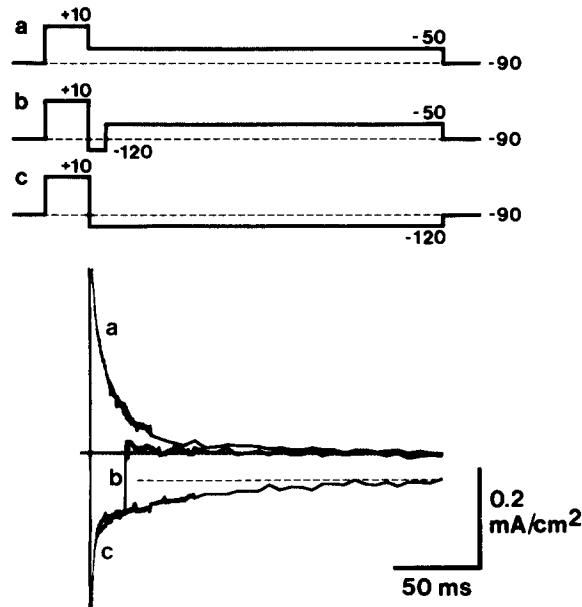


FIGURE 3. Rectification of slow tail. The currents shown in the lower panel of the figure were evoked by the pulse protocols shown in the upper panel. In each of the three protocols potassium channels were activated by a 25-ms conditioning pulse to $+10$ mV. (The outward current during the conditioning pulse is not shown.) Stepping voltage from $+10$ to -50 mV resulted in an outward tail of current (trace *a*), whereas stepping to -120 mV resulted in an inward tail (trace *c*). A slowly decaying component is clearly present in the tail current at -120 mV. After the conditioning pulse in protocol *b*, voltage was stepped to -120 mV for 20 ms and then stepped to -50 mV. Under the hypothesis that the slow tail at -120 mV represents the closing of channels that contribute to the total outward current during the conditioning pulse, an appreciable outward current should have been observed at -50 mV with pulse protocol *b*, since the slow component of the inward tail at -120 mV (trace *c*) decays very little in 20 ms. Current trace *b* demonstrates that this is not the case. Instead, it appears that the channels responsible for the slow inward tail of current at -120 mV rectify: they are capable of carrying inward current, but not outward. $T = 15^{\circ}\text{C}$. Muscle 71-2: $l, l' = 140, 70 \mu\text{m}$; $r_i = 7.8 \text{ M}\Omega/\text{cm}$; $\lambda = 674 \mu\text{m}$; $C = 14.1 \mu\text{F}/\text{cm}^2$.

of the outward tail in current trace *a*. The simplest conclusion from this result is that at -120 mV, all the channels responsible for delayed outward current in the omohyoid close during the fast tail, and that the slow kinetic component of the tail current at -50 mV represents a slow step in the closing of these same channels.

Rb Eliminates Anomalous Rectification and Slow Inward Tail Current

A further test of the hypothesis that the slow inward tail current is carried by the anomalous rectifier is illustrated in Figs. 4 and 5. (In both figures, total currents are shown without leak subtraction in order to better reveal the effects of anomalous rectification.) Fig. 4 demonstrates the presence of the anomalous rectifier in the omohyoid muscle. Thus, for currents recorded in normal K, hyperpolarizing the fiber by 30 mV resulted in a current almost twofold larger than depolarizing the fiber by 30 mV. As in frog muscle (Adrian, 1964; Adrian et al., 1970*b*), substitution of Rb for K eliminates this anomalous rectification. In addition to blocking the anomalous rectifier, Rb

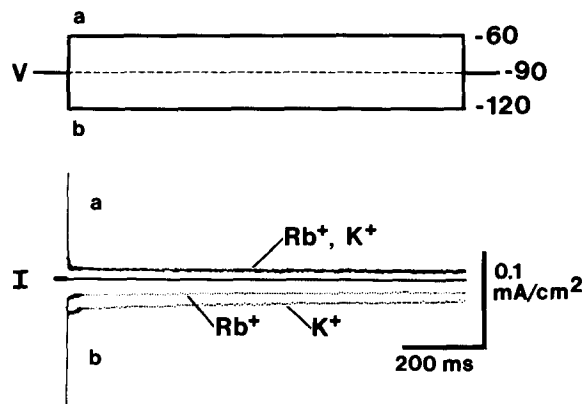


FIGURE 4. Rb blocks the anomalous rectifier. Upper panel: pulse protocols. Voltage was stepped from the holding potential (-90 mV) to either -60 mV (*a*) or -120 mV (*b*). The currents are shown in the lower panel. In the presence of extracellular K, the fiber displays anomalous rectification: the magnitude of the inward current at -120 mV is roughly two times the magnitude of the outward current at -60 mV. (The records in this figure are total currents with no correction for leak current.) Substitution of Rb for K has no effect on the outward current at -60 mV, but reduces the inward current at -120 mV so that its magnitude is nearly the same as that of the outward current. $T = 14.3^\circ\text{C}$. Muscle 52-2: for cable properties see legend to Fig. 2.

also alters the behavior of the tail currents seen after a conditioning pulse that activates delayed rectifier channels. Fig. 5 compares tail currents in K (middle portion of the figure) and Rb (lower portion of the figure) recorded at -120 mV after a conditioning pulse to $+10$ mV (traces labeled *b*). For comparison, currents at -120 mV without a prior conditioning pulse are also shown (traces labeled *a*). With potassium outside the fiber, the tail current following the $+10$ -mV conditioning pulse decays with a rapid phase (difficult to distinguish from the capacity transient) followed by a large slow tail. Switching from potassium to rubidium eliminated the slow tail but not the rapidly decaying phase (lower portion of Fig. 5).

Fig. 6 demonstrates that the time course of an outward current evoked by

a +10-mV test pulse is not affected by the replacement of K by Rb. The results for other test pulses examined (−40 to +30 mV) were similar. Thus, rubidium simplifies the kinetics of inward tail currents without affecting the time course of activation.

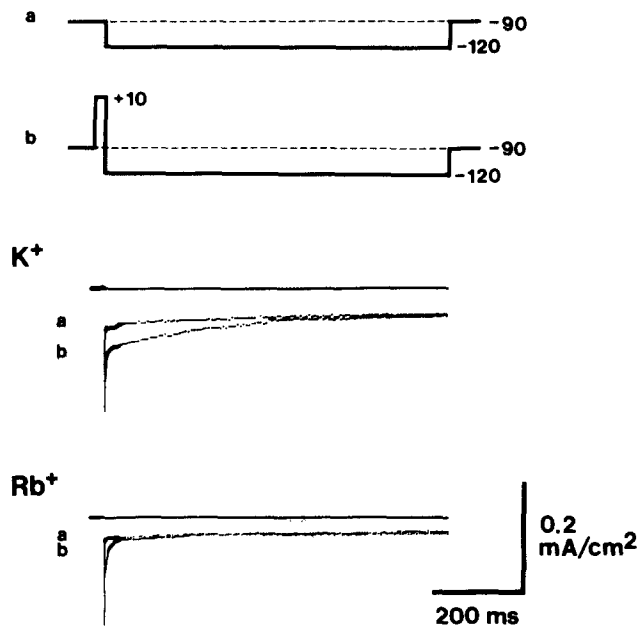


FIGURE 5. Rb eliminates the slow inward tail. Upper panel: pulse protocols. Membrane potential was stepped to -120 mV either directly from the holding potential (*a*) or after a 25-ms conditioning pulse to $+10$ mV (*b*). Middle panel: in the presence of extracellular K, the conditioning pulse causes the appearance of the slow tail (*b*). (The fast component of the tail, which is also a consequence of the conditioning pulse, is hard to see, because the time base is relatively slow and because the currents are shown without leak subtraction.) The slow inward tail decays to the level of the current elicited by a step directly to -120 mV without the conditioning pulse (*a*). Lower panel: after replacement of K in the bath by Rb the slow inward tail is absent and only the fast component of the tail remains. Thus, the tail current following the $+10$ -mV conditioning pulse (*b*) rapidly decays to the level of current seen in the absence of the prior conditioning pulse (*a*). Muscle 52-2: see Fig. 2.

To summarize, rubidium (*a*) blocks the anomalous rectifier, (*b*) abolishes the slow phase of inward tail currents, (*c*) does not abolish the fast tail, and (*d*) does not alter the activation kinetics of outward current. Effects *a* and *b* argue that the anomalous rectifier is responsible for the slow tail; effects *c* and *d* argue that all the channels responsible for delayed outward currents close during the rapid phase of inward tail currents. Both these conclusions are also supported by the results of the experiment illustrated in Fig. 3. Thus, the data are consistent with the hypothesis that a single class of potassium channels gives rise to delayed rectifier currents in the omohyoid.

Rubidium Hinders Potassium Channel Closing

Although the predominant effect of rubidium is to block the anomalous rectifier channel, the ion also has an effect on the delayed rectifier channel. In particular, close examination of Fig. 5 reveals that the fast component of inward tail current is slower with Rb outside the fiber than with K. This point is examined in greater detail in Fig. 7, which compares inward and outward tail currents before and after Rb substitution. The amplitude and time course of the outward current tail in rubidium were only slightly different from those of the tail measured in potassium at the same voltage. This similarity of outward tail currents at -50 mV is consistent with the similarity of activation kinetics seen for test pulses to -40 mV and above (e.g., Fig. 6).

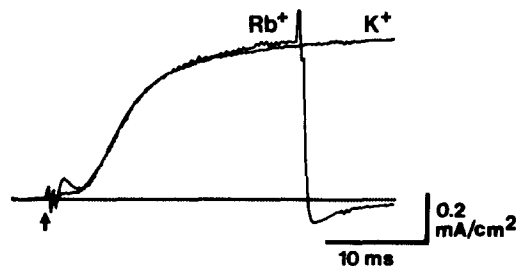


FIGURE 6. Rb does not affect activation kinetics. Currents elicited by test pulses to $+10$ mV. (The test pulse for the current recorded in Rb was terminated after 25 ms.) The arrow indicates the onset of the test pulses. $T = 14.3^{\circ}\text{C}$. Muscle 57-6: $l, l' = 154, 70 \mu\text{m}$; $r_i = 11.4 \text{ M}\Omega/\text{cm}$; $\lambda = 838 \mu\text{m}$; $C = 6.7 \mu\text{F}/\text{cm}^2$.

By contrast, Fig. 7 reveals that rubidium reduced both the amplitude and the decay rate of the fast component of the inward tail current. A similar effect has been reported for squid giant axon by Swenson and Armstrong (1981). They suggested that the closing of a potassium channel is hindered or prevented when Rb occupies a site within the channel and that Rb remains bound to this site longer than K. This mechanism could not only explain the slowing of the fast component's decay, but also the reduction of its magnitude, since if rubidium ions remained within the channel longer than potassium, the rubidium current would be smaller.

Fig. 8 gives a quantitative comparison between the degree to which rubidium reduces the amplitude of tail currents and the degree to which it slows their decay. Tail currents were measured using a standard two-pulse protocol in which test pulses ranging from -120 to -50 mV were applied to the fiber at the termination of a conditioning pulse to $+10$ mV. Each tail current was fitted with a single exponential; the time constant of this exponential was used as the measure of decay rate, and the magnitude of the exponential, extrapolated to the start of the test pulse, was used as the measure of amplitude. For outward currents the exponential was fit to the entire tail, whereas for inward currents recorded in potassium the slow component of the tail was mathematically subtracted from the record before fitting. This procedure assumes

that all of the outward tail represents delayed K channels closing, whereas only the fast component of inward tails represents such closing. The Rb slowing of K channel closing is plotted as open squares in Fig. 8. The degree of slowing increases with hyperpolarization so that at -50 mV, rubidium causes the channels to close with a rate constant ~ 0.75 that seen with potassium outside, whereas at -120 mV the rate constant was reduced to only ~ 0.4 that measured in potassium. This variable degree of slowing is expected under the hypothesis that Rb must bind to a site within the channel in order to interfere with channel closure. Since Rb is only present outside the cell, the concentration of Rb within the channel would be expected to be much higher

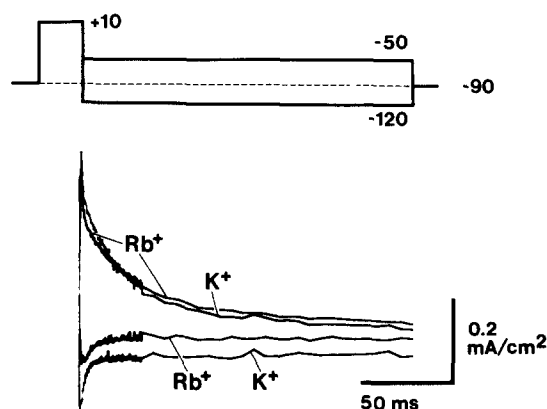


FIGURE 7. Effect of Rb on fast tail kinetics. Upper panel: pulse protocols. After a 25-ms pulse to $+10$ mV, voltage was stepped to either -50 or -120 mV. Lower panel: currents recorded before and after substituting Rb for extracellular K. Rb has relatively little effect on either the amplitude or time course of the outward tail current at -50 mV, but reduces the amplitude and the rate of decay of the inward tail current at -120 mV. Note that Rb also reduces the steady-state current at -120 mV by blocking the anomalous rectifier. $T = 14.3^{\circ}\text{C}$. Same fiber as in Fig. 2.

at hyperpolarized potentials when current is inward than at more depolarized levels when current is outward.

Swenson and Armstrong's (1981) hypothesis implies that the rubidium-induced reduction of tail current amplitude provides a separate measure of channel occupancy by rubidium and this measure should have a voltage dependence similar to that of the rubidium-induced slowing of channel closing. Fig. 8 demonstrates that a similar voltage dependence is in fact observed. The reduction in tail current amplitude was quantified by comparing the conductance of the channel just before (g_{cond}) and just after (g_{tail}) the step from the conditioning to the test level. The rationale for using this measure is that during the conditioning pulse the driving force for current flow is strongly outward so that the occupancy of the channel by rubidium should have been negligible. A justification of this procedure is provided by

the observation described earlier, that delayed outward currents are the same in Rb as they were in K (Fig. 6). The data of Fig. 8 are consistent with the hypothesis that the reduction of tail current amplitude (filled circles) and the slowing of tail current decay (squares) are related and that both are a consequence of the entry of Rb into the channel. A more detailed quantitative analysis of these data is probably unwarranted because even though rubidium replaced potassium in the bulk medium, outward current flow during the

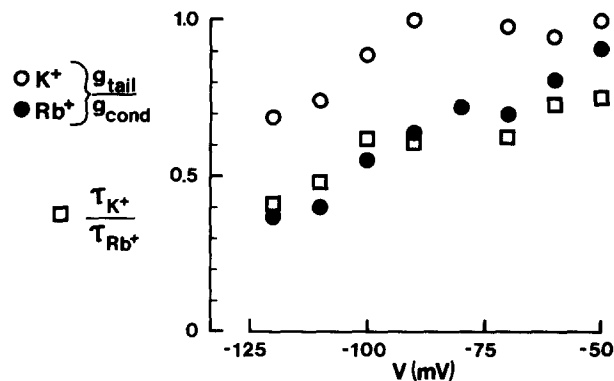


FIGURE 8. Rb causes a parallel rectification and slowing of the fast tail's decay. After a 25-ms conditioning pulse to +10 mV, the fiber was repolarized to the test levels that are plotted along the abscissa. The resulting tail currents were measured before and after Rb substitution. The circles give the ratio between g_{tail} , the conductance of the outward tail or fast inward tail just after the conditioning pulse, and g_{cond} , the conductance at the end of the conditioning pulse. This ratio was measured with the fiber bathed in K (filled circles) and in Rb (open circles). The squares give the ratio between the time constant of the fast tail before (τ_K) and after (τ_{Rb}) substitution of Rb for K. Time constants were determined by fitting a single exponential to the tail current (only the fast component of inward tail in K was fit; the slow component was taken as baseline). The fitted exponential was projected back to the time of the step from the conditioning level to the test level in order to determine the quantity I_{tail} . The conductances were then calculated according to the expressions $g_{cond} = I_{cond}/(V - V_K)$, $g_{tail} = I_{tail}/(V - V_K)$, where V_K is the reversal potential of tail current (-80 for the run in K^+ and -85 for the run in Rb^+), and I_{cond} is the magnitude of the current at the end of the conditioning pulse. Data are from the same fiber as in Fig. 7.

conditioning pulse undoubtedly caused some potassium accumulation in the immediate vicinity of the fiber.

The open circles in Fig. 8 plot the quantity g_{tail}/g_{cond} for currents recorded with potassium outside the fiber, where g_{tail} for inward tails is the conductance of the fast phase only. For an ohmic channel, the ratio g_{tail}/g_{cond} should be unity for all test potentials. This was approximately the case at -90 mV and above, but for more hyperpolarized potentials the ratio falls off from unity somewhat. Although it is possible that the channel rectifies at strongly

hyperpolarized potentials, it seems more likely that the voltage clamp was too slow to accurately resolve tails at such potentials.

DISCUSSION

The simplest interpretation of our results is that the channels responsible for delayed K current in the omohyoid are of a single kind that closes during the fast component of inward tail current. The slow component of inward tail current appears to result from potassium accumulating during outward currents in a restricted extracellular space. This accumulated potassium causes an increase in current through the anomalous rectifier and the decay of this increased current is manifest as the slow inward tail. A number of observations support this conclusion. The existence of an accumulation space is argued by the dependence of V_K on the duration of outward current flow. The behavior of the slow tail is qualitatively consistent with buildup and removal of K from a restricted space: the slow tail is larger and decays more slowly when it follows a prolonged outward current than when it follows a brief one, and the decay of the tail has a weak temperature dependence. The pathway responsible for the slow tail displays properties consistent with the anomalous rectifier: outward tail currents do not possess a slow component that obviously corresponds to the slow inward component. Moreover, Rb simultaneously blocks the anomalous rectifier and the slow inward tail. Thus, the existence of distinct fast and slow phases of inward tail current does not appear to constitute evidence for the existence of two different kinds of delayed K channel.

Two experimental observations support the idea that the slow kinetic component of outward tail currents arises from a different mechanism than the slow inward tail. The first is that the channels responsible for the slow inward tail are incapable of carrying appreciable outward current (Fig. 3). Thus, these channels cannot contribute to the slow kinetic component of outward tails. The second is that Rb eliminates the slow inward tail (Fig. 5), but not the slow kinetic component of outward tail currents: a single exponential is not sufficient to fit either of the tail currents at -50 mV in Fig. 7, whether recorded in K or recorded in Rb. This result argues that the slow inward tail in potassium arises from a separate channel species (the anomalous rectifier), whereas the closing of delayed rectifier channels at depolarized potentials is inherently multi-exponential, perhaps because of recovery from an inactivated state (cf. Eq. 11 of Beam and Donaldson, 1983). By contrast, at hyperpolarized potentials, channel closing appears to be dominated by a single rapid exponential, since a 20-ms hyperpolarization to -120 mV reduces the conductance of the delayed rectifier to a negligible level (Fig. 3).

The suggestion that a single kind of delayed K channel is present in the omohyoid is supported by the observation that for fibers bathed in potassium the ratio of the fast inward tail conductance to the conductance at the end of the conditioning pulse ($g_{\text{tail}}/g_{\text{cond}}$) remained close to unity even at potentials where the total tail current shows both rapid and slow phases of decay (Fig. 8). If the fast component of decay did not represent the closing of all the channels that were contributing to the outward current during the condition-

ing pulse (i.e., the current from which g_{cond} was calculated), then one would expect the conductance calculated from the fast tail to be less than the conductance calculated from the outward current at the end of the conditioning pulse. Put another way, the difference between $g_{\text{tail}}/g_{\text{cond}}$ and unity should be a measure of the contribution to the total outward current by channels that do not close during the fast phase of the tail current (assuming a linear instantaneous I - V relation for all the channels contributing to the outward current). Pursuing this line of reasoning, if we consider the tail current at -100 mV illustrated in Fig. 3, $g_{\text{tail}}/g_{\text{cond}}$ was 0.89 (Fig. 8), which suggests that at most 11% of the outward current was carried by channels not closing during the fast phase. For voltages negative to -100 , $g_{\text{tail}}/g_{\text{cond}}$ was farther from unity, but as discussed earlier, it seems likely that this represents experimental artifact. Thus, any slow K channels that are present in the omohyoid appear to contribute little to delayed outward currents for the pulse durations examined here (25–100 ms). Alternatively, if a second kind of K channel is present, then it must close with kinetics similar to those of the delayed rectifier.

Although only a single kind of delayed rectifier channel seems to be present in the omohyoid, other muscles appear to possess more than one. Thus, based on kinetic and pharmacological evidence, both fast and slow K channels are present in frog sartorius muscle (Adrian et al., 1970*b*; Stanfield, 1970) and frog toe muscle lumbricalis digiti IV (Lynch, 1978). In the case of toe muscle, the relative number of slow channels varies considerably from fiber to fiber. A predominantly slow-twitch mammalian muscle, the soleus of the rat, also appears to possess at least two distinct delayed K channels (Duval and Léoty, 1980*b*). In contrast, the data argue against the presence of more than one delayed K channel in rat iliacus muscle (Duval and Léoty, 1980*b*). Since the iliacus and the omohyoid are both predominantly fast-twitch, the results presented in this paper, together with those of Duval and Léoty (1980*b*), support the generalization that mammalian fast-twitch muscle contains a single kind of delayed rectifier, and that the slow tail is a consequence of K accumulation.

The morphological identity of this hypothetical accumulation space remains unclear. The calculated volume of the space is sufficiently large to suggest that the bulk of delayed rectifier channels must be on or near the surface of the fiber. In particular, the volume of the accumulation space is too large in both the omohyoid (Table I) and in the frog sartorius (Adrian et al., 1970*a*) to be that of the transverse tubules. The same applies to the accumulation space calculated for the soleus and iliacus from the data presented in Fig. 6C of Duval and Léoty (1980*b*), for fiber diameters assumed to be in the range 50–100 μm (cf. Duval and Léoty, 1980*a*). In calculating this volume, the simplifying assumption is made that during the flow of outward current potassium enters the restricted space but does not leave it. As long as τ , the time constant for emptying the restricted space, is long compared with T , the duration of the outward current, the error introduced by this assumption is small; if τ is less than T , then the calculated volume is too large by a factor of

about T/τ . Two observations suggest that for T on the order of 100 ms, the calculated volume should not have been seriously in error. First, τ cannot be very much shorter than 100 ms, otherwise the tail current reversal potential would be different at short times after the repolarizing step than at longer times. Such behavior is not seen (Fig. 2). The second is that the slow tail, which according to the accumulation hypothesis reflects the removal of K from the restricted space, has a time constant on the order of 100 ms.

The volume of the restricted space is closer to that of the T system plus sarcoplasmic reticulum (SR) than that of the T system alone, but there are several arguments against the SR with its larger volume being part of the restricted space. In the first place, even with the inclusion of the SR, which in fast-twitch mammalian muscle occupies $\sim 5\%$ of the total fiber volume (Luff and Atwood, 1971; Eisenberg and Kuda, 1975), the composite volume of the T system plus SR is less than half that calculated for the restricted space. Moreover, if one is to consider the entire volume of the SR as contributing to the restricted space, then it is necessary to further suppose that on the time scale of the measured shifts in V_K , potassium is able to diffuse between the SR and T system. In turn, the free diffusional exchange of potassium between these two compartments implies an electrically conductive pathway between them that would cause the capacitance of the SR to appear electrically in parallel with the surface of the fiber. If this latter were the case, the very large surface area of the SR (Eisenberg and Kuda, 1975) would cause total fiber capacitance to be much larger than actually measured (Beam and Donaldson, 1983, and references therein). An additional argument that the lumen of the SR does not function as a component of the extracellular space is provided by recent electron-probe measurements of frog muscle which demonstrate that the K content of the SR is like that of the myoplasm (Somlyo et al., 1981). Thus, potassium exiting the fiber through K channels would have to enter the SR against its electrochemical gradient. Under the assumption that mammalian muscle is similar, it seems simplest to conclude that the SR does not participate in the K accumulation space and that this space is instead a consequence of the presence of basement membrane, connective tissue, and neighboring fibers (in whole-muscle experiments). The dimensions of this restricted compartment could be quite modest: a cylindrical annulus 1.5 μm thick would have a volume 12% that of a 50- μm fiber.

Duval and Léoty (1980*b*) have reported that glycerol shock eliminates the slow inward tail in rat iliocostalis muscle, but it is by no means clear that this effect is a result of detubulation. For example, one could suppose that detubulation largely eliminates the anomalous rectifier channels, which we postulate are responsible for the slow tail. One would expect detubulation in frog muscle to eliminate the bulk of anomalous rectification (Almers, 1980), but in rat muscle this does not appear to be the case (Palade, 1976). As an alternative, therefore, one could suppose that detubulation eliminates potassium accumulation. Consistent with this suggestion, glycerol shock reduces the time-dependent shift of V_K (Duval and Léoty, 1980*b*) in both soleus and iliocostalis. However, even if the tubular system is a component of the accumu-

lation space, its relative contribution appears to be so small that eliminating it should not matter. Thus, the effects of glycerol shock on slow tail currents in rat skeletal muscle appear to be unrelated to any effect of detubulation. Instead, it may be that glycerol shock causes not only detubulation but also breaks down the diffusion barrier presented by the basement membrane and connective tissue.

A standard method for measuring the time course of activation of putative slow K channels has been to measure the amplitude of the slow inward tail as a function of the duration of the prior conditioning pulse (Adrian et al., 1970*b*; Duval and Léoty, 1978, 1980*a, b*). Assuming it is correct, however, that potassium accumulation causes the slow inward tail in fast-twitch rat muscle, it seems likely that potassium accumulation would also give rise to a slow component of inward tail current in muscles that actually do possess slow K channels. This "accumulation tail" would be expected to contaminate any "true" slow tail that actually reflected the closing of slow channels. One way to estimate the contribution of accumulation would be to measure slow tail currents as a function of conditioning pulse duration in the presence of rubidium. Assuming Rb is permeant through slow K channels, such an experiment would allow determination of the activation kinetics of slow K channels. Fig. 14 of Adrian et al. (1970*b*) illustrates the current obtained when a frog muscle bathed in 10 mM Rb was depolarized to -11 mV and then repolarized to -137 mV. An inward tail current is present, but the absence of a time calibration makes it difficult to determine whether it has kinetics appropriate to a slow K channel or whether it represents the closing of ordinary delayed rectifier channels which has been slowed by rubidium. Thus, the contribution of accumulation to the slow tails measured by Adrian et al. (1970*b*) is unclear. A useful alternative, which allows an estimate of accumulation without relying on the permeability of slow K channels for Rb, is to use pulse protocols like the one illustrated in Fig. 3 (trace *b*).

In summary, there appears to be a real difference in the number of distinct kinds of potassium channels present in different skeletal muscle fiber types. For now, one can only speculate as to the reason why some muscles possess slow K channels and others do not. It is possible that slow K channels serve an unidentified but important physiological function. Alternatively, such channels may be vestigial and may have been physiologically important only at an earlier evolutionary or ontogenetic stage.

We wish to thank Dr. D. Campbell for advice during the work and comments on the manuscript.

This work was supported by the Muscular Dystrophy Association and NS14901.

Received for publication 24 May 1982 and in revised form 27 September 1982.

REFERENCES

- Adrian, R. H. 1964. The rubidium and potassium permeabilities of frog muscle membrane. *J. Physiol. (Lond.)*. 175:134-159.
- Adrian, R. H., W. K. Chandler, and A. L. Hodgkin. 1970*a*. Voltage clamp experiments in striated muscle fibers. *J. Physiol. (Lond.)*. 208:607-644.

- Adrian, R. H., W. K. Chandler, and A. L. Hodgkin. 1970b. Slow changes in potassium permeability in skeletal muscle. *J. Physiol. (Lond.)*. 208:645-668.
- Almers, W. 1980. Potassium concentration changes in the transverse tubules of vertebrate skeletal muscle. *Fed. Proc.* 39:1527-1532.
- Barrett, J. N., E. F. Barrett, and L. B. Dribin. 1981. Calcium-dependent slow potassium conductance in rat skeletal muscle myotubes. *Dev. Biol.* 82:258-266.
- Beam, K. G., and P. L. Donaldson. 1983. A quantitative study of potassium channel kinetics in rat skeletal muscle from 1 to 37°C. *J. Gen. Physiol.* 81:485-512.
- Donaldson, P. L., and K. G. Beam. 1981. Does mammalian fast twitch skeletal muscle possess two delayed rectifier channels? *Soc. Neurosci. Abstr.* 7:903.
- Duval, A., and C. Léoty. 1978. Ionic currents in mammalian fast skeletal muscle. *J. Physiol. (Lond.)*. 278:403-423.
- Duval, A., and C. Léoty. 1980a. Ionic currents in slow twitch skeletal muscle in the rat. *J. Physiol. (Lond.)*. 307:23-41.
- Duval, A., and C. Léoty. 1980b. Comparison between the delayed outward current in slow and fast twitch skeletal muscle in the rat. *J. Physiol. (Lond.)*. 307:43-57.
- Eisenberg, B. R., and A. M. Kuda. 1975. Stereological analysis of mammalian skeletal muscle. II. White vastus muscle of the adult guinea pig. *J. Ultrastruct. Res.* 51:176-187.
- Luff, A. R., and H. L. Atwood. 1971. Changes in the sarcoplasmic reticulum and transverse tubular system of fast and slow skeletal muscles of the mouse during postnatal development. *J. Cell Biol.* 51:369-383.
- Lynch, C. 1978. Kinetic and biochemical separation of delayed rectifier currents in frog striated muscle. *Biophys. J.* 21:55a. (Abstr.)
- Müntener, M., J. Gottschall, W. Neuhuber, A. Mysicka, and W. Zenker. 1980. The ansa cervicalis and the infrahyoid muscles of the rat. *Anat. Embryol.* 159:49-57.
- Palade, P. T. 1976. Perturbations of chloride conductance in a mammalian skeletal muscle preparation: the rat diaphragm. Ph.D. Thesis, University of Pennsylvania, Philadelphia, PA.
- Pallotta, B. S., K. L. Magleby, and J. N. Barrett. 1981. Single channel recordings of Ca activated K currents in cultured rat muscle cells. *Nature (Lond.)*. 293:471-474.
- Somlyo, A. V., H. Gonzalez-Serratos, H. Shuman, G. McClellan, and A. P. Somlyo. 1981. Calcium release and ionic changes in the sarcoplasmic reticulum of tetanized muscle: an electron-probe study. *J. Cell Biol.* 90:577-594.
- Stanfield, P. R. 1970. The effect of the tetraethylammonium ion on the delayed currents of frog skeletal muscle. *J. Physiol. (Lond.)*. 209:209-229.
- Swenson, R. P., and C. M. Armstrong. 1981. K channels close more slowly in the presence of external K and Rb. *Nature (Lond.)*. 291:427-429.